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LABORATORY-SCALE STUDY IN DETERMINING THE DECONTAMINATION STANDARDS FOR PERSONNEL PROTECTIVE EQUIPMENT USED BY HOMELAND DEFENSE PERSONNEL: EVALUATION OF COMMERCIAL OFF-THE-SHELF TECHNOLOGIES FOR DECONTAMINATION OF PERSONNEL PROTECTIVE EQUIPMENT-RELEVANT SURFACES

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14. ABSTRACT

In 2001, dissemination of letters tainted with anthrax-causing spores through USPS led to a limited number of deaths and contamination of several hundred thousand cubic feet of surface space within government and commercial buildings. In the context of homeland security, these and other cases involving ricin toxin demonstrated the urgent need to develop countermeasures for cleaning up complex surfaces relevant to personnel protective equipment (PPE). Four relevant surface materials (polycarbonate, steel, Tyvek®, and butyl rubber) were intentionally contaminated with either 10E7 spores of avirulent ΔSterne *Bacillus anthracis* or 50 μg ricin protein toxin. Two disinfection technologies, pH-adjusted dilute Clorox® bleach and dilute Peridox™, were tested for their ability to decontaminate within 15 min of contact time. The results from this study showed that >6 log kill of spores and near-complete decontamination of ricin toxin were observed within 15 min treatment with dilute Clorox. Dilute Peridox resulted in complete spore kill, but was not effective in the decontamination of ricin protein even after 30 min treatment. Based on the results of this lab-scale study, recommendations for decontamination standards for PPE used by first responders will be discussed.

15. SUBJECT TERMS

ΔSterne *Bacillus anthracis*	Tyvek	Butyl rubber
Stainless steel	Polycarbonate	Clorox
Peridox	PPE	CT

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PREFACE

The work described in this report was authorized under the Project titled Laboratory-Scale Study Determining the Decontamination Standards for Personnel Protective Equipment Used by First Responders. The work was started in November 2006 and completed in January 2008.

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1. INTRODUCTION

Since the anthrax incidents following the tragic events of September 2001, the prospect of another biological warfare (BW) event has been a major concern and has served as an impetus for biodefense research. This project was funded by the Department of Homeland Security (DHS) through an Inter-Agency Agreement (IAA) with the National Institute of Standards and Technology (NIST). The Standards Development Team was concerned with the decontamination standards for the personnel protective equipment (PPE) used by Homeland Defense personnel. Such personnel are called upon following a BW event, risking exposure in the hot zone. Consequently, decontamination of PPE is vital for restoration and re-use. In this report, decontamination of PPE surfaces contaminated with two common BW agents, *Bacillus anthracis* spores and ricin toxin, was investigated using commercial off-the-shelf (COTS) technology.

Decontamination of *Bacillus* species on different surface types has been an active area of research in recent years. The decontamination efficacy of chlorine dioxide gas and vaporous hydrogen peroxide on anthrax contaminated interior surfaces of a building has been previously investigated by Rastogi *et al.*, 2007 and Wallace *et al.*, 2005. In the two previous studies, small-size coupons prepared from cinder block, pinewood, ceiling tile, wallboard, I beam steel, and carpet were used (Rastogi *et al.*, 2007). Decontamination efficacy was dependent on three critical factors, the surface type, CT (concentration in ppm multiplied by exposure time in hours) or dose of the fumigant, and relative humidity (RH).

The overall objective of the present study was to investigate the effectiveness of two disinfectants on the following four surfaces:

- Tyvek
- Butyl rubber
- Stainless steel
- Polycarbonate

Tyvek® is the material used for the protective suits worn by Homeland Defense personnel. Butyl rubber is common to the respirator masks used by first responders. Stainless steel is common to the equipment and vehicles used by Homeland Defense personnel. Finally, polycarbonate is relevant to the goggles used by Homeland Defense personnel.

Small-size samples (coupons) of these materials were inoculated with either 10^7 of plasmid-free, avirulent *B. anthracis* spores (ΔSterne), or 50 μg ricin protein toxin, a non-

replicating BW agent. The contaminated coupons were then disinfected with either pH adjusted bleach or dilute Peridox™ (Clean Earth Technology).

2. METHODS AND MATERIALS

2.1 Bacterial Strains, Culture Conditions, Toxin Procurement, and Disinfectants

The plasmid-free, avirulent strain of *B. anthracis*, ΔSterne, was obtained from the Department of Defense Unified Culture Collection (culture no. BAC1056). The spores were cultured on trypticase soy agar (TSA) plates at 37 °C and then processed according to an in-house spore preparation protocol. The spore suspension was heat-treated and ethanol-treated to rid the sample of vegetative cells. Microscopic analyses after spore staining indicated the presence of >90% spores. The purified (>90%) ricin toxin was procured from Vector Laboratories and then diluted ($1/10^{th}$) to prepare the working stock.

The disinfectants used in this project were Clorox® bleach which is ~5.6% or 48,000 ppm and Clean Earth Peridox™. Peridox™ is supplied as a concentrate containing 1-1.4% peroxyacetic acid (CAS #79-21-0), 24-25% hydrogen peroxide (CAS # 7722-84-1), and 1-1.4% acetic acid. Clorox® bleach was diluted $1/10^{th}$ with sterile distilled water. Clean Earth Peridox™ was diluted $1/6^{th}$ with sterile distilled water. The disinfectants were used within 2 hr of their preparation.

2.2 Coupon Procurement

Small size coupons (2^2 cm each) were procured from commercial vendors and then cut by the Edgewood Chemical Biological Center machine shop. The steel was 16-Ga, hot rolled steel commercial quality, and conformed to ASTM A415. The impermeable Tyvek® (item # LL-01SH) was purchased from Weiss Brothers. The polycarbonate was 0.177 ft thick and was purchased from EJ Enterprises and the butyl rubber (item # 8609K35) was purchased from McMaster Carr. The coupons were sterilized in Petri plates by autoclaving for 15 min using a dry cycle prior to use.

2.3 Inoculation of Coupons

The coupons were inoculated with either 50 µL containing 10^7 spore of avirulent anthrax (ΔSterne) or 50 µL of 1 mg/mL of pure ricin toxin. Five spots of 10 µL each of either ricin or spores were inoculated on each coupon. The coupons were dried in a biological safety cabinet overnight.

2.4 Recovery Experiments

Recovery of *B. anthracis* spores or ricin toxin from coupons was investigated as a prelude to the disinfection studies. The spore inoculated coupons were dropped into a 50 mL falcon tube containing 10 mL recovery media consisting of 0.5% buffered peptone water (BPW) plus 0.01% Tween 80. A titer control was performed by adding 50 µL of the spore stock directly

into the 10 mL recovery media. The tubes were sonicated for 10 min and then vortexed for 2 min. A ten-fold serial dilution was done followed by plating 100 µL aliquots on TSA plates in triplicate. The plates were incubated in a 37 °C incubator for 24 hr, and then colony-forming units (CFUs) were counted using a Qcount™ plate counter. The number of viable spores from the control coupons was computed by multiplying the average CFU from triplicate plates with volume factor (10) and dilution factor.

% Recovery = [Total CFU recovered/number of spores inoculated] x 100

The ricin inoculated coupons were dropped into a 50 mL falcon tube containing 10 mL recovery media. Additionally, a positive control sample was set up by aliquoting 50 µL ricin directly into 10 mL recovery media. Twenty µL of each protein sample was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Sypro stain to visualize the protein bands. The dynamic range of the Sypro sensitivity is semi-quantitative, i.e. approximate quantification range can be determined by comparing the intensity of the control ricin samples with that of the staining intensity observed in test samples.

2.5 Peridox™ and Clorox® as Disinfectants against ΔSterne

The effect of a 15 min exposure of appropriately diluted disinfectants on spore contaminated coupons was performed in 50 mL sterile tubes. The coupons were dropped into 7.5 mL of disinfectant and after a 15 min contact time, 2.5 mL of 2 M sodium thiosulphate ($Na_2S_2O_3$) was added to neutralize the disinfectant. The coupons were then treated the same as the control samples, as stated in section 2.4.

2.6 Peridox™ and Clorox® as Disinfectants against Ricin Protein

The effect of a 15 min Clorox® contact on ricin protein was investigated, as was the effect of a 30 min Peridox™ contact on ricin protein. The coupons were decontaminated by dropping them into 7.5 mL of disinfectant, after the 15 min or 30 min, 2.5 mL of sodium thiosulphate was added to neutralize the disinfectant. The samples were then treated as controls as detailed in section 2.4. The absence of ricin protein bands in treated samples was concluded to be indicative of effective decontamination by a given technology.

3. RESULTS

3.1 Recovery Experiments

The control sample (no treatment) numbers were used to determine the recovery of ΔSterne spores from four surface types, and to ensure suitability of the extraction process for each surface type. As seen in Figure 1, the spore recovery from each of the four surfaces was between 70-90%.

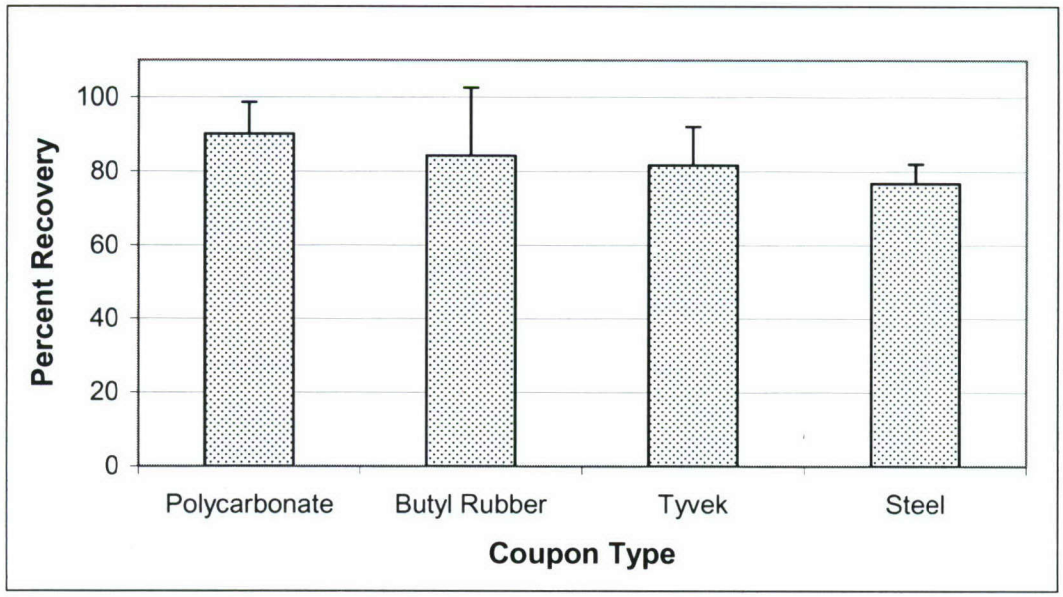

Figure 1. Percent Recovery of ΔSterne Anthrax Spores from PPE Surfaces

Recovery experiments were also performed for ricin protein. The recovery of ricin was determined by SDS-PAGE and Sypro staining. Two bands on the gel indicate that ricin was recovered from the coupon surfaces as shown in Figures 2a and 2b.

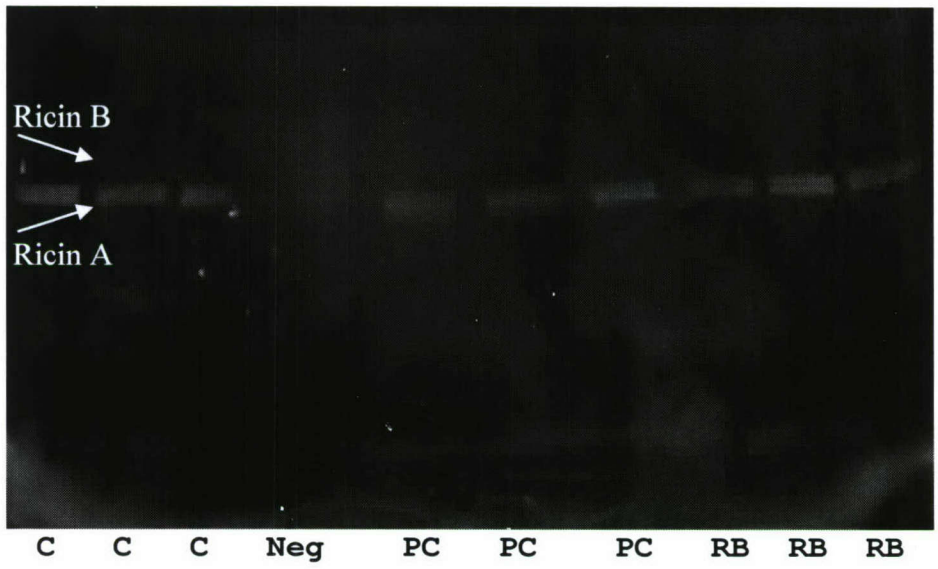

Figure 2a. Electrophoretic Separation of Ricin Protein on SDS-PAGE: Polycarbonate and Butyl Rubber Coupons

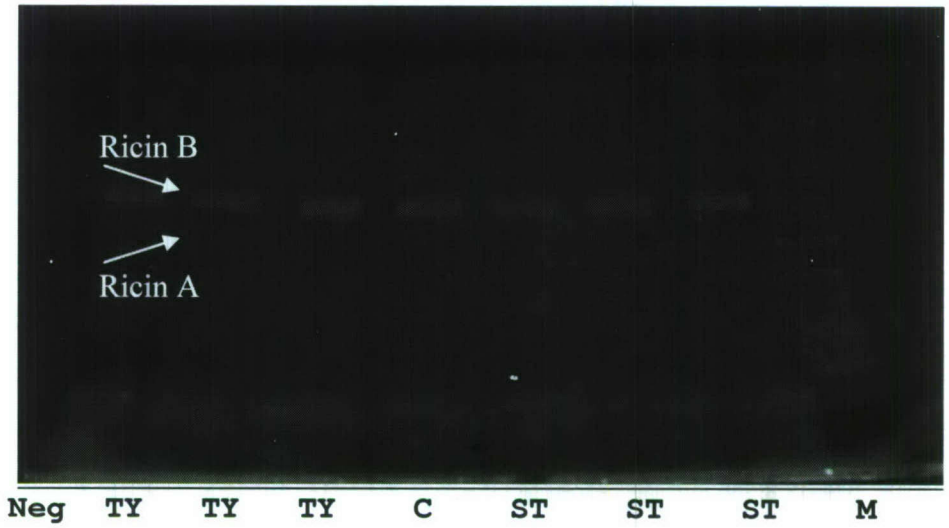

Figure 2b. Electrophoretic Separation of Ricin Protein on SDS-PAGE: Tyvek® and Steel Coupons

C=Control; PC= polycarbonate; RB=butyl rubber; TY=Tyvek®; ST=Steel; M=Protein Marker

3.2 Peridox™ and Clorox® as Disinfectants against ΔSterne Spores

No viable spores were recovered from any of the four surfaces after a 15 min treatment with dilute Peridox™. As seen in Figure 3, this treatment resulted in >6 log spore for each surface type. A similar level of spore kill was observed with 1/10th Clorox® after a 15 min contact time (Figure 4).

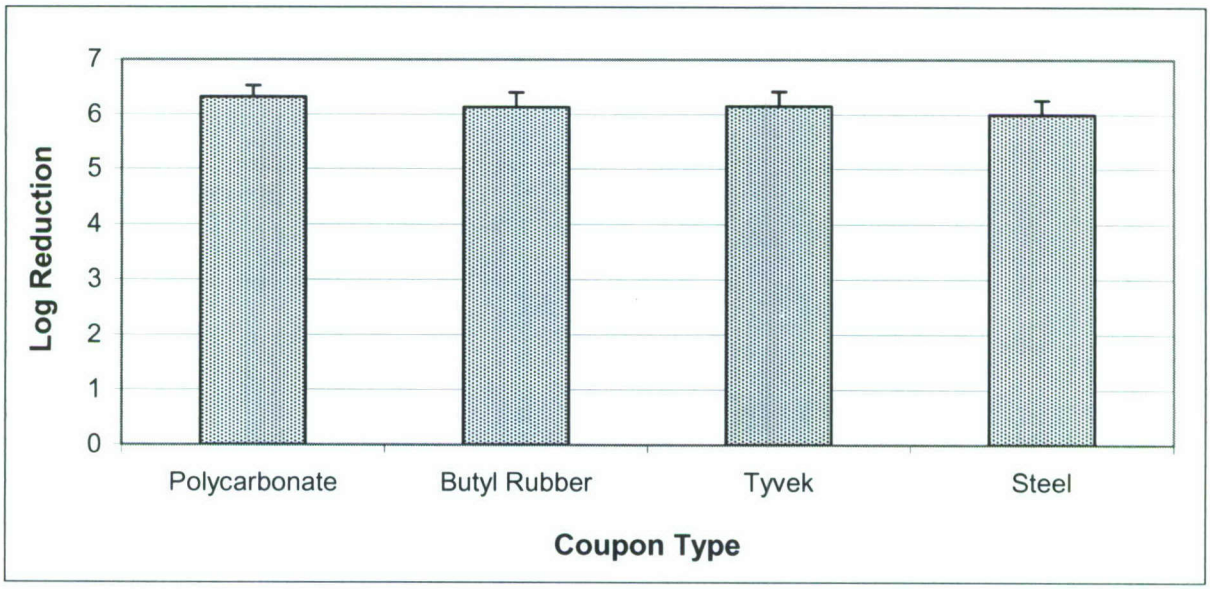

Figure 3. Log Reduction of Avirulent Anthrax Spores Following 15-min Contact with 1/6th Diluted Peridox™

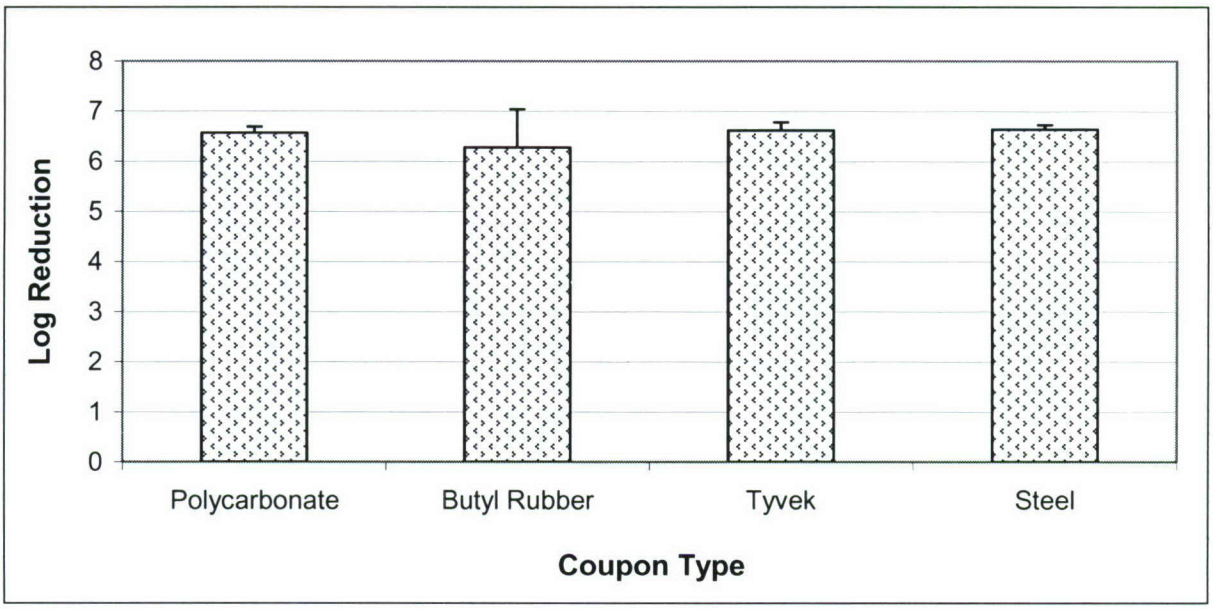

Figure 4. Log Reduction of Avirulent Anthrax Spores Following 15 min Contact with 1/10th Diluted Clorox® (pH 7)

3.3 Clorox® and Peridox™ as Disinfectants against Ricin Protein

Ricin was not detected in samples recovered from polycarbonate, steel, Tyvek® , or butyl rubber after 15 min treatment with dilute Clorox® , as seen in Figures 5a and 5b. Ricin was detected after 30 min contact time with dilute Peridox™ for all four sample types, as seen in Figures 6a and 6b. Positions of ricin A and B are indicated by the arrows and can be seen in each lane except negative samples (Figures 6a and 6b).

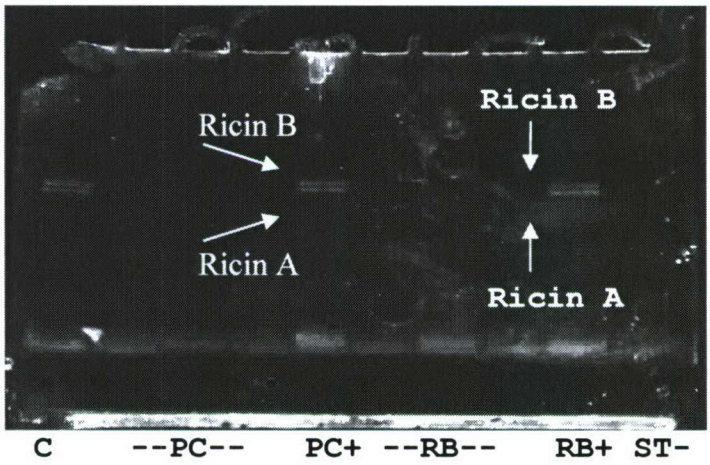

Figure 5a. Electrophoretic Separation and Detection of Ricin in Clorox® Disinfected Samples from Polycarbonate and Butyl Rubber

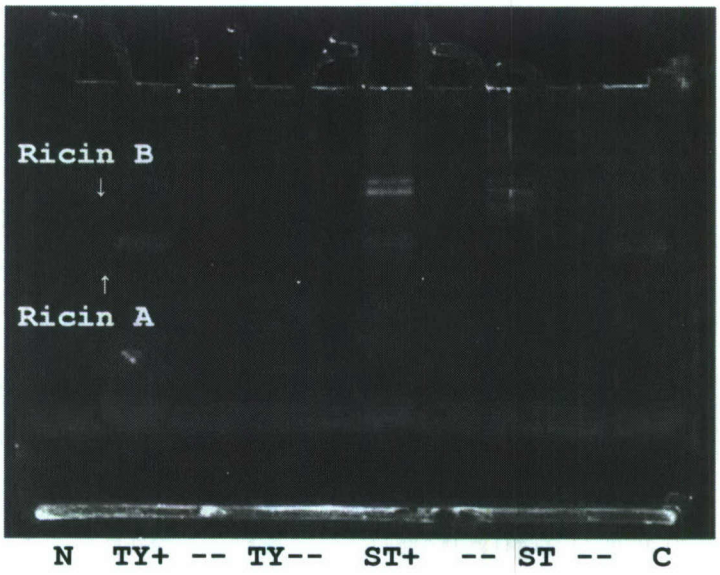

Figure 5b. Electrophoretic Separation and Detection of Ricin in Clorox® Disinfected Samples from Tyvek® and Steel

N = neg. control;
RB+, ST+, TY+, and PC+ = positive controls;
C = ricin control;
--PC-- = polycarbonate test coupons treated with Clorox®;
--RB-- = butyl rubber test coupons treated with Clorox®;
--ST-- = steel test coupons treated with Clorox®;
--TY-- = Tyvek® test coupons treated with Clorox®;
Positions of ricin A and B are indicated by the arrows.

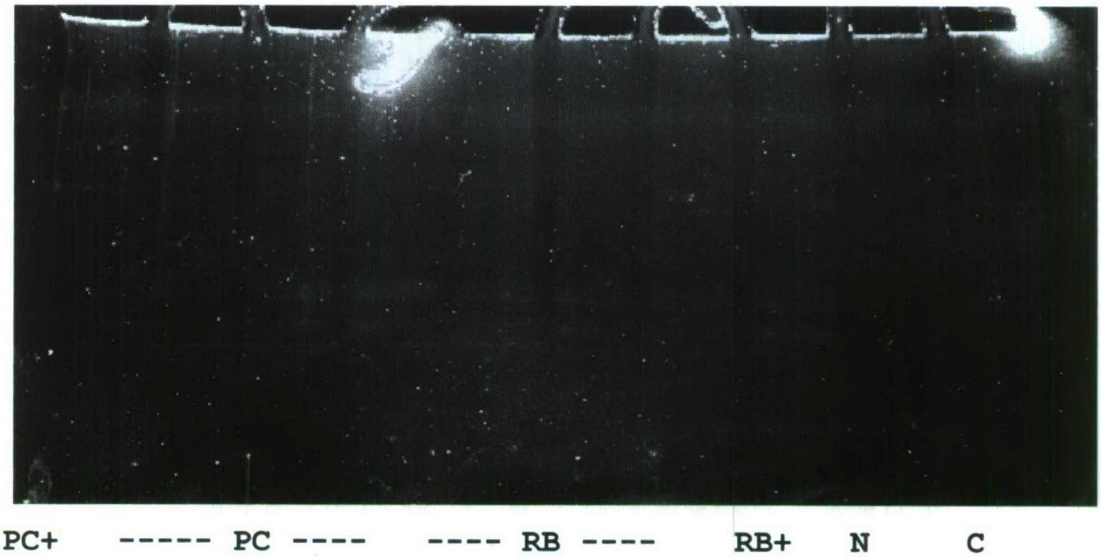

Figure 6a. Electrophoretic Separation and Detection of Ricin in Peridox™ Disinfected Samples from Polycarbonate and Butyl Rubber

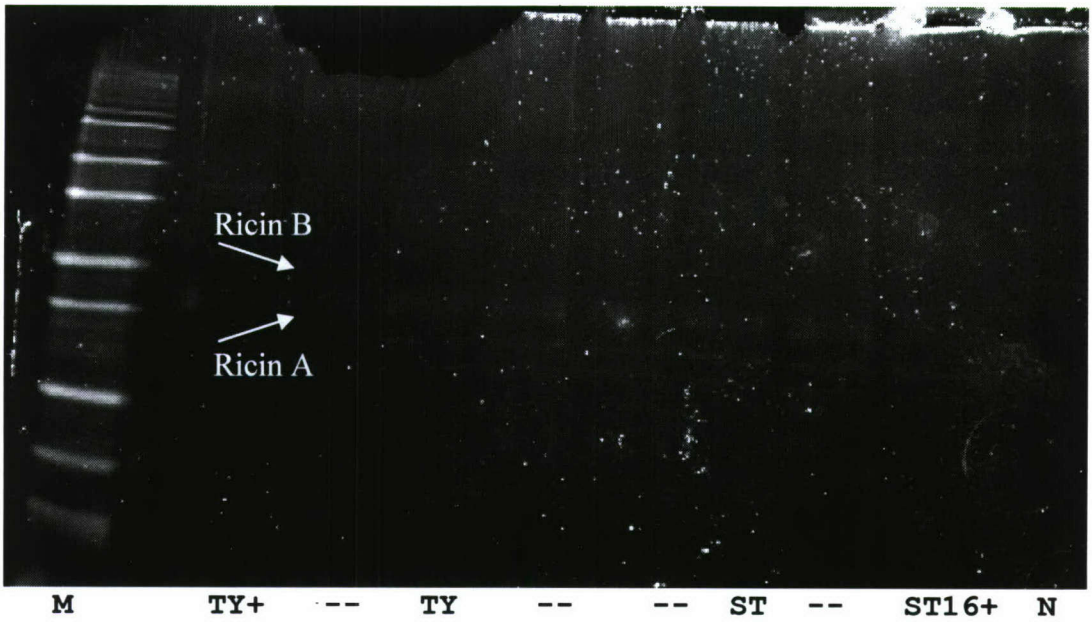

Figure 6b. Electrophoretic Separation and Detection of Ricin in Peridox™ Disinfected Samples from Tyvek® and Steel

N = neg. control;
RB+, ST+, TY+, and PC+ = positive controls;
C = ricin control;
--PC-- = polycarbonate test coupons treated with Peridox™;
--RB-- = butyl rubber test coupons treated with Peridox™;
--ST-- = steel test coupons treated with Peridox™;
--TY-- = Tyvek® test coupons treated with Peridox™.
Positions of ricin A and B are indicated by the arrows.

4. DISCUSSION AND CONCLUSIONS

High recovery (>50%) of biological contaminants from environmental surfaces is pivotal to quantitative efficacy studies with disinfectants and log reduction estimations. In this study, since 70-90% of the inoculated spores were recovered from all four PPE-relevant surfaces, the extraction procedures used here are adequate for conducting decontamination studies. Reported values for spore recoveries for *Bacillus anthracis* Sterne spore ranged between 43%, from steel coupons (Rose et al., 2004), to 31% from galvanized metal (Rogers *et al.*, 2005). In this study, estimated recovery using the procedures described in Materials and Methods is between 70-90%.

Two commercial technologies, Peridox™ and Clorox®, were tested for their sporicidal effectiveness in decontamination of surfaces with a 15 min or 30 min contact time. Complete spore kill on steel, Tyvek®, and polycarbonate coupons was observed with both technologies. Complete spore kill was observed on butyl rubber after Peridox™ treatment, but ~6 log kill was observed with Clorox® treatment. In a recent study, 5% bleach was shown to decontaminate *B. atrophaeus* on protective suit material and metal with a 30 min contact time (Kenar *et al.*, 2007). In another study, a 6.7 log reduction in *B. anthracis* ΔSterne dried on rubber

and metal was observed following Clorox® treatment for a 30 min contact time (Sagripanti et al., 2006). The results for avirulent *B. anthracis* are consistent with the published results. These results indicate that Peridox™ and Clorox® are effective disinfectants for decontaminating the PPE that are used by the Homeland Defense personnel.

Based on the findings in this study, Clorox® would be an effective disinfectant for decontaminating ricin protein on all four surfaces tested as ricin was not detected following a 15 min contact time. The limit of detection of ricin on SDS-PAGE is 10-20 ng of ricin subunits, while the LD_{50} for ricin in laboratory mice is 3-5 μg/kg by inhalation, 5 mg/kg by intravenous injection, and 20 mg/kg via intra-gastric ingestion. Conversely, Peridox™ would not be a good disinfectant since ricin was detected even after a 30 min contact time.

Effective decontamination of PPE and other equipment used for Homeland Defense personnel can be achieved by 15- to 30-min treatment with $1/10^{th}$ dilute Clorox®. The acceptable standards for decontamination should be zero viable spores, since the infectious dose for anthrax spores range between one and several thousands, depending on a variety of factors, such as age, sex, immunity, and other health conditions. Even though no ricin protein activity was detected on SDS-PAGE, further confirmation of loss of biological activity must be demonstrated. More work is therefore required in confirming the absence of biologically active ricin toxin following decontamination with bleach.

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